HIV-1 Tat: Structure and Function

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Introduction

Most viruses encode functions for regulating genome transcription. Examples include the SV40 T-antigen, the adenovirus E1a protein, and the herpes virus immediate-early proteins. For the human immunodeficiency viruses (HIV), Tat functions similarly, though not identically, to those other activators. Over the past decade, we have learned much about Tat, both in structure and in function. The intent of this brief accompanying overview is to "add flavor" to raw data, it is written for the purpose of apprising, in a short format, the readers on some of the current thoughts about Tat. For more in-depth discussions, extensive subject reviews can be found elsewhere (e.g. Jones and Peterlin, 1994; Chang et al., 1995; Gatignol et al., 1996). This overview was revised from an earlier version appearing in the 1994 compendium.

Tat function

Tat is a small nuclear protein of 86 to 101 amino acids (depending on the viral strain) which is encoded from two separate exons (see Section I). Analyses of "full-length" Tat have been performed commonly using the 86 amino acid version. However, it should be noted that while a few laboratory strains (e.g. HXB2 and NL4–3) have the truncated Tat (86 aa) most HIV-1s have the 101 aa protein (see compendium part II).

A. LTR Transcription. Despite intensive efforts, the mechanism of Tat action remains incompletely understood. It is accepted that Tat is required for optimal HIV viability (Fisher et al., 1986; Dayton et al., 1986). The role of Tat in critically directing transcription (Peterlin et al., 1986; Rice and Mathews, 1988; Laspia and Mathews, 1989) from the HIV LTR is one necessary function suggested for this protein. However, increasingly there is evidence that Tat has other significant effects on the virus and on the host cell (Huang et al., 1994; Chang et al., 1995; Neuveut and Jeang, 1996; Goldstein, 1996). In the setting of viral pathogenesis, both aspects need to be considered.

In activating transcription from the LTR, Tat differs from other prototypic viral trans-activators in requiring a bipartite responsive element consisting of DNA and RNA. To our knowledge, Tat is the first characterized eukaryotic transcription factor that binds to a nascent leader RNA, TAR (Berkhout et al., 1989; Dingwall et al., 1989; Cordingley et al., 1990; Roy et al., 1990; Calnan et al., 1991); and then influences events at the TATAA-enhancer-promoter (Berkhout et al., 1990; Selby and Peterlin, 1990; Southgate et al., 1990; Jeang et al., 1993b; Gatignol et al., 1996; see fig. 1A). TAR RNA has an extensive secondary structure including a stem, a bulge, and a loop (Muesing et al.,1987; Berkhout and Jeang, 1989; Roy et al., 1990c; Wang and Rana, 1996; see fig. 1B). Early studies indicated that the UCU sequence of the bulge is critical for binding by Tat (Dingwall et al. 1989,; Roy et al. 1990a, Calnan et al., 1991; Cordingley et al., 1990). By contrast, the structure of the stem, but not its specific sequence, was proposed to be important for function. The loop of TAR RNA serves as binding site(s) for cellular factor(s) that cooperate with Tat in the activation of the LTR (Sheline et al., 1991; Wu et al., 1991). A more extensive discussion of the role of cellular factors that bind TAR RNA (Gatignol et al., 1989; Gaynor et al., 1989; Gatignol et al., 1991; Gatignol et al., 1996) is presented in Section IV.

Both for Tat binding and for transcription, there are sequence specific requirements for the immediate stem nucleotide pairs that flank the bulge (Weeks and Crothers, 1991; Berkhout and Jeang, 1991; Churcher et al., 1993; Wang et al., 1996). In a recent series of studies, Rana and colleagues have provided physical insights on how Tat interacts with TAR RNA. Using photo-activated cross-linkers, they were able to elucidate the orientation of Tat as it intercalates into TAR RNA. They found that

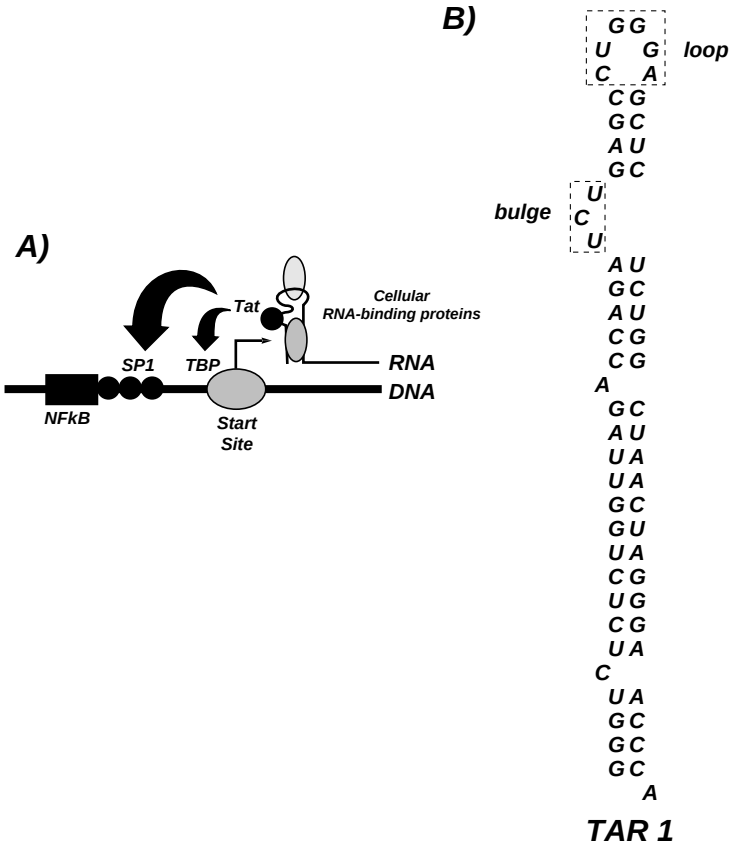


Fig. 1. Interaction of Tat with DNA and RNA targets in the HIV-1 LTR. A) A schematic representation of the functional interactions between Tat, TAR-RNA-binding proteins and promoter elements. Biochemical evidence exists that Tat contacts directly SP1 (Jeang et al., 1993b) and TATAA-binding protein (TBP; Kashanchi et al., 1994). B) Secondary structure of TAR RNA. The crucial trinucleotide bulge and hexanucleotide loop elements are boxed.

amino acid 41 of Tat lies in close proximity to U42 in the lower TAR stem, that amino acid 47 of Tat is proximal to the G26 nucleotide positioned immediately above the TAR bulge, and that amino acid 57 of Tat is close to U31 in the TAR loop (Wang and Rana, 1995; Liu et al., 1996; Wang et al., 1996). At the same time, the basic domain of Tat was shown to interact with RNA residues U23, U38, and U40 and to distort/widen the major groove of TAR RNA (Wang and Rana, 1996; Metzger et al., 1996).

While it is clear that Tat binds TAR RNA and interacts with enhancer-promoter-binding factors (Berkhout and Jeang, 1992; Jeang et al., 1993a; Southgate and Green, 1995), the direct mechanism through which these physical events influence transcription is not wholly evident. A number of models that attempt to explain the transcription function of Tat have been proposed. These include i) anti-terminating (Kao et al., 1987) stalled RNA polymerase II (RNAP II), ii) increasing processivity/elongation of transcribing RNAPII complex (Laspia et al., 1989; Marciniak et al., 1990; Hermann and Rice, 1995; Zhou and Sharp, 1996), and iii) facilitating formation of RNAPII complexes at the promoter (Laspia et al., 1989, Jeang and Berkhout, 1992; Jeang et al., 1993a; reviewed in Cullen, 1993). Currently, there are findings consistent with each of these models. Possibly, Tat, like basal transcription factor TFIIF (Buratowski, 1994), acts simultaneously as an initiation and elongation factor. Alternatively, because transcription from a given promoter is a dynamic multi-cycled process, efficient

disassembly (i.e. processive promoter-clearance) of a previous round is necessary to permit initiation of the next round (reviewed in Zawel and Reinberg, 1995). Thus, initiation and elongation/clearance events at the promoter may be mechanistically linked and, in some cases, inseparable processes. Recent findings indicate that Tat plays a mechanistic role not at the stage of recruiting TBP to the promoter (Kashanchi et al., 1994), but at the step of clearing RNA polymerase II from the promoter (Chun and Jeang, 1996; Xiao and Jeang, unpublished observations).

Lastly, one must exercise care in not assuming that different look-alike experimental systems would share the same the rate-limiting step (Jeang et al., 1993a). Two examples suggest caution. First, results from Pomerantz and co-workers have recently shown that short LTR-transcripts are not found in cells that are fully functional for Tat-transactivation (Niikura et al., 1996). Second, some of the analogies drawn between EIAV Tat and HIV-1 Tat bear re-visiting. One recent report has raised the consideration that EIAV Tat is a DNA-binding and not an RNA-binding protein (Rosch and Willbold; 1996).

B. Translation. There are suggestions that Tat also functions in regulating translation (Rosen et al., 1986; Cullen, 1987). TAR RNA can inhibit translation (Parkin et al., 1988; SenGupta and Silverman, 1989) of HIV-1 mRNAs, most likely through activation of double-stranded RNA-dependent protein kinase (PKR) and 2–5A synthetase (SenGupta and Silverman, 1989; Edery et al., 1989). Addition of Tat was found to reverse this translational inhibition (SenGupta et al., 1990; Braddock et al., 1990). It is possible that one part of Tat's translational effect stems from its ability to form physically a functional complex with PKR (McMillan et al., 1995).

C. Effects on cellular function. There is increasing evidence that Tat has pleiotropic effects on cellular genes and host cell metabolism (reviewed in Chang et al., 1995). This would not be surprising, since it clearly serves the advantage of the virus to be able to modify optimally the cellular environment for replication. In this regard, Tat has been reported to function as a secreted growth factor in stimulating the growth of Kaposi-like cells (Ensoli et al., 1990; Ensoli et al., 1993; Barillari et al., 1993) and in promoting angiogenesis (Albini et al., 1996). Tat is further described to affect the organization of neurons and astrocytes (Kolson et al., 1993); it is neurotoxic at low concentrations (Sabatier et al., 1991).

Expression of many cytokines is modulated by Tat (Rautonen and Rautonen, 1992). These include TNFa,b (Buonogaro et al., 1992; 1994), TGFa,b (Lotz et al., 1994; Nabell et al., 1994), IL-2 (Purvis et al., 1992; Westerndorf et al., 1994), and IL-6 (Scala et al., 1994) among others. Tat has also been reported to activate cellular signal transduction pathways that involve phosphatidylinositol-3-kinase (Milani et al., 1996) and NF-kB (Biswa et al., 1995). lt has been further suggested that Tat can affect programmed cell death by protecting lymphocytes against apoptosis (Gibellini et al., 1995). However, the validity of this last point point has been contested by conflicting findings that Tat promotes apoptotic death of lymphocytes (Li et al., 1995).

Domains in Tat

Tat is synthesized from an mRNA joined from two coding exons. The first exon encodes amino acids 1–72 and (in most strains of HIV-1) the second exon encodes amino acids 73–101 (see fig. 2). In reporter plasmid co-transfection assays, the first 72 amino acids of Tat fully trans-activates transcription from the LTR. In fact, a truncated 58 amino acid form of Tat is virtually wild type in this type of co-transfection assay (Seigel et al., 1986; Garcia et al., 1988; Kuppusway et al., 1989). Whether data from plasmid co-transfections reflect accurately the normal physiological function of Tat during replication of HIV-1 in lymphocytes has been a poorly studied issue. Recent investigations suggest that reporter plasmid co-transfection results cannot be interpreted literally for their relevance towards viral replication (Neuveut and Jeang, 1996).

A. First coding exon. The combined results from many laboratories have permitted an arbitrary demarcation of "domains" in Tat (Kuppuswamy et al., 1989). For instance, the N-terminus of Tat

(domain 1; fig. 2) has 13 amino acids with amphipathic characteristics. Mutations that alter the acidic composition of this region were felt originally to affect trans-activation (Rappaport et al., 1989); however, results from a later study conflicted with this interpretation (Tiley et al., 1990).

Amino acids 22 to 37 (domain 2, fig. 2) contain seven cysteines and are highly conserved between different isolates of HIV-1s. Individual mutation in six of the seven cysteines abolish Tat function (see Table I). Although originally proposed as a metal-chelating dimerization domain (Frankel et al., 1988), this region was recently shown to be used for intra-molecular disulfide bond formation in monomeric molecules of Tat protein found inside cells (Koken et al., 1994). Currently, it is believed that Tat is active functionally as a monomer rather than a dimer (Rice and Chan, 1991; Koken et al., 1994).

Domain 3 (amino acids 40 to 48) contains a RKGLGI motif that is conserved between HIV-1, HIV-2 and SIV Tat. This region, in conjunction with the amino terminus and the cysteine domain, has been suggested to circumscribe the minimal activation domain of HIV-1 Tat (Carroll et al., 1991; Derse et al., 1991). Domain 4 (amino acids 49–72) contains a basic RKKRRQRRR motif. These amino acids confer TAR RNA-binding properties to Tat (Dingwall et al., 1989; Roy et al., 1990; Weeks et al., 1990; Chang and Jeang, 1992) and are important for nuclear localization of the protein (Ruben et al., 1989; Hauber et al., 1989). However, recent studies suggest that this short basic stretch is insufficient in determining the entire specificity of Tat-TAR binding since amino acids outside of the basic domain also contribute to this interaction (Churcher et al., 1993; Luo et al., 1993).

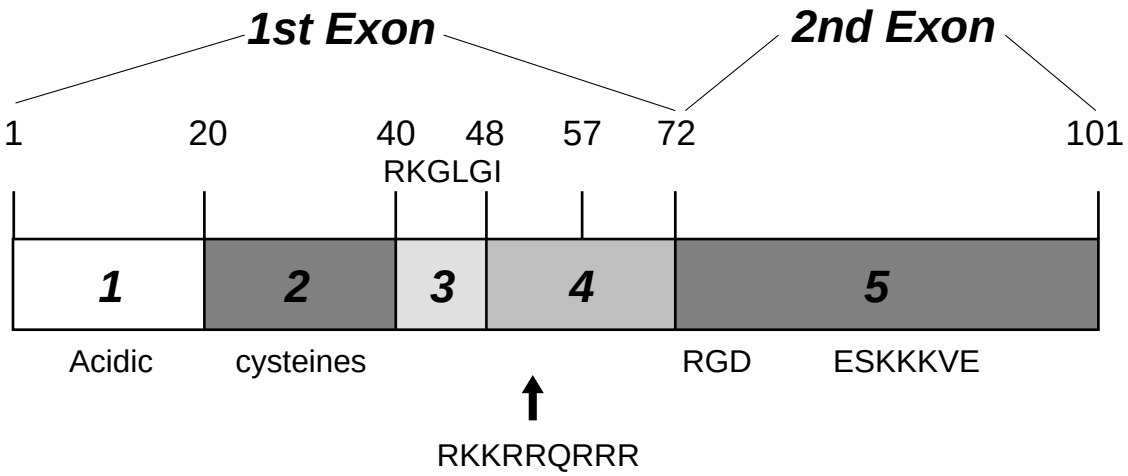


Fig. 2. Domain classifications of Tat protein. The demarcation of domains is somewhat arbitrary. The first exon includes amino acids 1–72, while the second exon includes 73–101. Motifs and characteristics of each "domain" are indicated above or below each region.

Table I summarizes 77 point mutations in Tat collated from the work of nine laboratories (Garcia et al., 1988; Sadaie et al., 1989; Kuppuswamy et al., 1989; Ruben et al., 1989; Hauber et al., 1989; Meyerhans et al., 1989; Rice and Carlotti, 1990a; Rice and Carlotti, 1990b; Siderovski et al., 1992; Neuveut and Jeang, 1996). As alluded to above, most studies were performed based on measurements from reporter-plasmids. Because the first 58 amino acids of Tat recapitulate well the trans-activation function in this type of assay, it is not surprising that the great majority of engineered mutations is concentrated within amino acids 1 to 58. In many cases, individual amino acids have been changed to more than one counterpart, heightening the validity of the resulting phenotype. In rare instances, mutational analysis of the second coding exon of Tat has been studied. Two recent point mutants of the second exon of Tat (P81fs and K90R) showed unexpectedly that changes in this exon perturb not measurements of plasmid trans-activation but do affect virus replication (Neuveut and Jeang, 1996).

Table I. Point mutations in Tat

Amino Acid Changes			Consensus
From	To	Activities	Amino Acid
Q2	A	++	E
P3	A	++	P
P3	Q	++	P
V4	A	++	V
D5	A	+	D
P6	A	++	P
P6	S	++	P
Δ3–6		+	
P6P10	LL	++	
R7	A	++	R
L8	A	++	L
E9	A	++	E
P10	A	++	P
P10P13	LL	++	
W11	A	++	W
K12	A	++	K
K12	N	++	K
P18	A	++	P
K19	R	++	K
A21	D	++	A
A21T23	VA	+	
T23	A	++	T
C22	S	–	C
C22	G	–	C
N23	T	++	T
N24	A	++	N
N24	K	++	N
C25	R	–	C
C25	G	–	C
Y26	A	+	Y
Y26	F	++	Y
C27	S	–	C
C27	G	–	C
K28K29	AA	+	KK
K28K29	EA	–	KK
C30	G	–	C
C31	S	++	C
C31	E	–	C
C31	G	++	C
F32	A	+	F
H33	A	–	H
C34	G	–	
C34	S	–	C
G35	A	+	Q
C37	G	–	C
C37	S	–	C

Table I. (cont) Point mutations in Tat

Amino Acid Changes From	To	Activities	Consensus Amino Acid
F38	A	$-$	F
F38	L	$++$	F
K40	D	$-$	T
K40	T	$++$	T
K41	A	$-$	K
*K41	T	$-$	K
*K41	T	$++$	K
L43	F	$+$	L
G44	S	$++$	G
S46	A	$++$	S
S46	P	$-$	S
Y47	H	$++$	Y
Y47	A	$++$	Y
G48	S	$++$	G
G48R49SG[1]		$++$	
R49	T	$++$	R
K50	stop	$-$	K
K50K51	Y50Y51	$+$	KK
K50K51	S50G51	$+/\pm$	KK
K50	E	$++$	K
K50	T	$++$	K
R52	E	$++$	R
R53	I	$++$	R
Q54	N	$++$	Q
R55	G	$+$	R
R55R56	L55T56	$+$	
R56	E	$++$	R
R57Q63	SE	$++$	RQ
L69	I	$++$	L
P81	fs[2]	$-$[3]	P
K90	R	$-$[3]	K

Column 1: First letter indicates original amino acid. Number indicates position of amino acid in Tat.

Column 2: Letter(s) indicate the resulting amino acid.

Column 3: $++ >$ 50% wild type activity; $+ >$ 10% wild type activity; $+/-$ or $-$ indicate $<$ 10% wild type activity.

Column 4: Because not all isolates of HIV-1 have the same amino acids for Tat, a consensus sequence is listed also.

Notes: * Different results reported for the same mutation from Kuppuswamy et al., 1989; and Meyerhans et al., 1989. [1]Amino acids beyond position 59 completely changed. [2]fs = frame shift of amino acids beyond position 81. [3]Measurement is based not upon trans-activation of a reporter plasmid but on delayed replication of an HIV-1 molecular clone in T-cell lines (Neuveut and Jeang, 1996).

Examination of the mutants (Table 1) reveals that the region spanning amino acids 1–21 is remarkably tolerant of changes. In contrast, changes in amino acids 22 through 40 were generally deleterious for trans-activation. Finally, although the basic domain (amino acids 49–57) as a unit is necessary for Tat function, individual amino acid changes do not significantly affect activity.

B. Second coding exon. The second coding exon of Tat has been less studied. In routine transfection of reporter plasmids, absence of the second exon does not alter greatly measurements of Tat activity in this type of assay. However, findings from HIV-2 and SIV Tat are quite clear in demonstrating that this exon contributes towards optimal trans-activation (Viglianti and Mullin, 1988; Tong-Starksen et al., 1993). There are emerging findings that the second exon of HIV-1 Tat, in other assays, is important for trans-activation (Jeang et al., 1993b), trans-repression (Howcroft et al., 1993), and virus replication (Neuveut and Jeang, 1996).

Two short motifs in the second exon of HIV-1 Tat could have functional importance (see fig. 2). The first is an RGD sequence that is used as a cell adhesion signal for binding to cellular integrins (Brake et al., 1990). This RGD motif , however, is not found in HIV-2 or SIV Tat proteins. Recently, it has been suggested that the basic domain of Tat (rather than the RGD sequence) is the important protein portion for cellular uptake (Albini et al., 1996). The second exon also has an ESKKKVE motif which is conserved in most HIV-1 Tat proteins and is partially preserved in HIV-2 and SIV Tats. The functional significance of this motif has not been examined in detail.

C. Immunological epitopes in Tat. Evidence suggests that Tat has a role in viral infectivity and can contribute to pathogenesis (Huang et al., 1994; Neuveut and Jeang, 1996). Indeed, in tissue culture experiments, findings suggest that the addition of antibody against Tat to the culture supernatant can halt the spread of HIV-1 infection in T-lymphocytes (Steinaa et al., 1994; Re et al., 1995). Complementary observations in patients indicate that immune response to Tat correlates with better prognosis for disease progression (Reiss et al., 1991). These results have led to the suggestion that Tat should be considered as a candidate prophylactic vaccine (Goldstein, 1996) for HIV-1.

Although the immunological epitopes within Tat have not been studied in detail, some information is available. Figure 3 summarizes findings from 4 studies. Two regions of Tat (amino acids 1–9 and 70–83) have been found to be potent in eliciting humoral responses. Three other regions have been described to elicit CTLs. Interestingly, it has been observed that uninfected individuals have natural IgM antibodies directed against two portions of Tat, suggesting that this might be one basis for natural immunity against the virus.

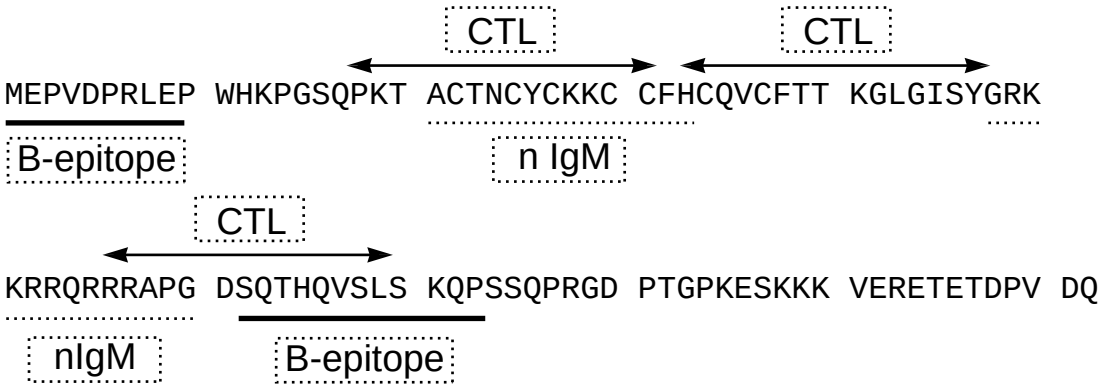
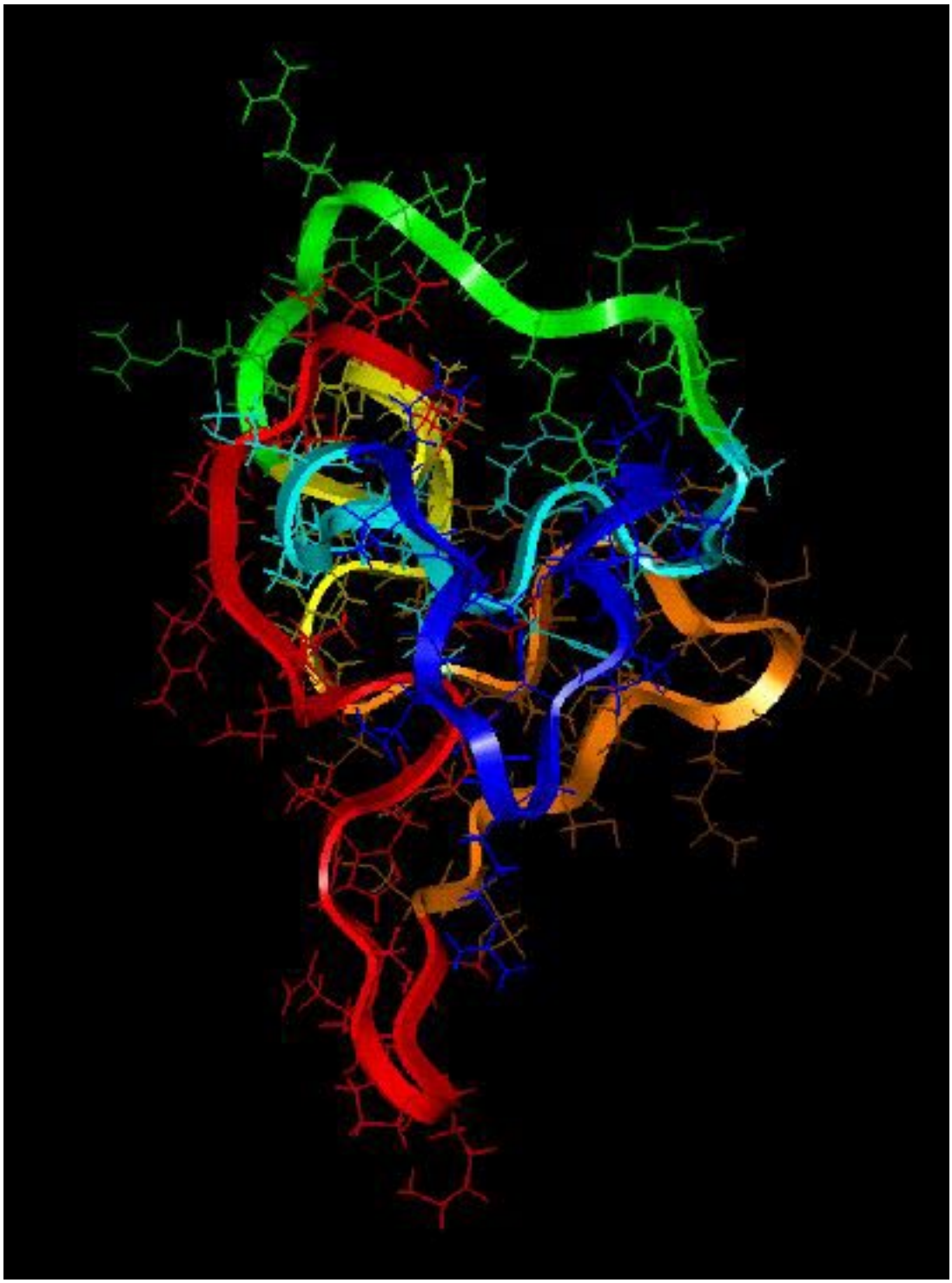


Fig. 3. Immunological epitopes in the HIV-1 Tat protein. B-cell immunodominant epitopes were determined by Krone et al. (1988) and McPhee et al. (1988); CTL-epitopes were mapped by Blazevic et al. (1993); and naturally occurring IgM epitopes in Tat were as reported by Rodman et al. (1993).

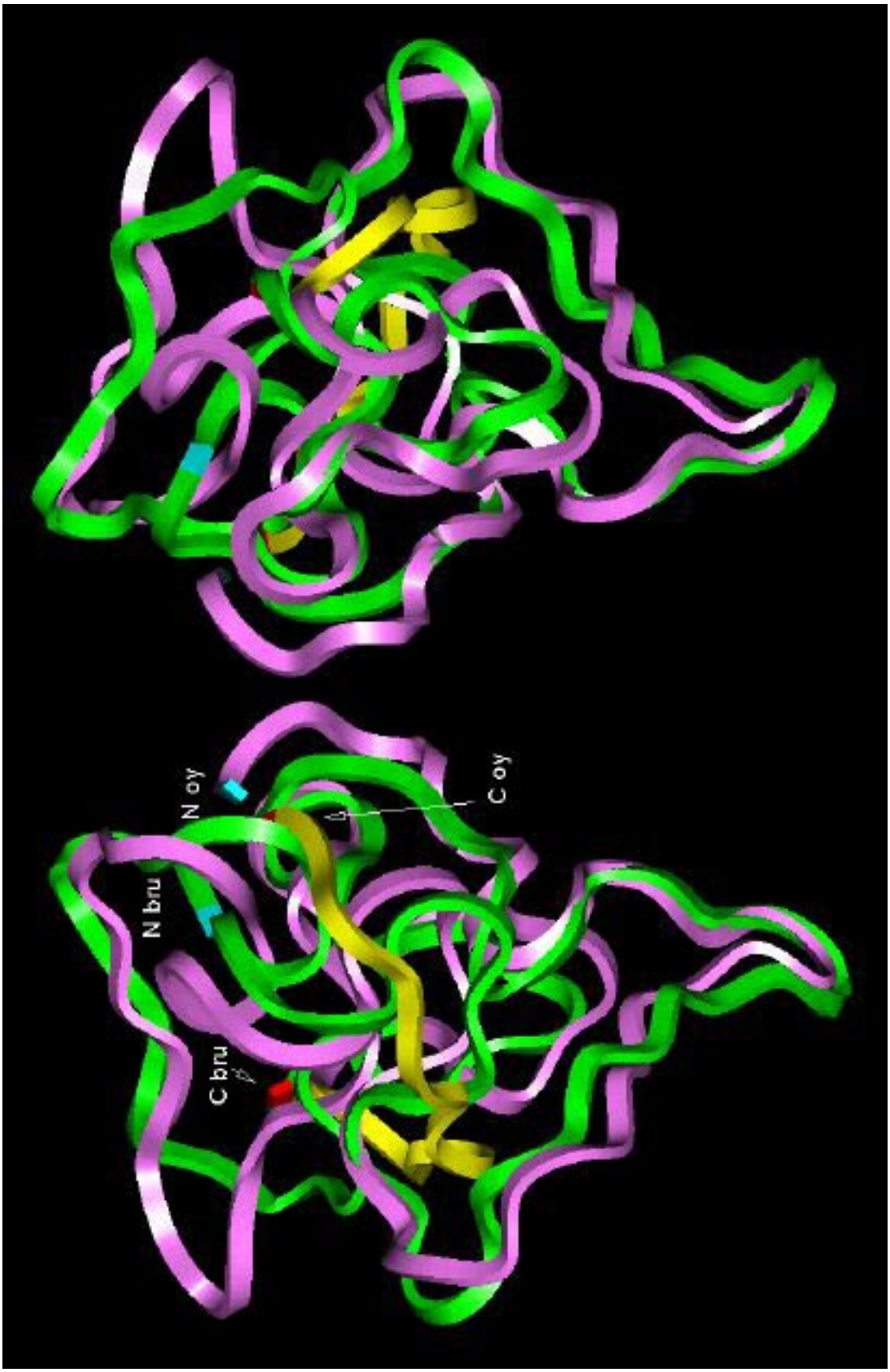
Concluding Remarks

For a relatively small protein, much effort has been expended on studying Tat. A recent search of Medline (conducted Nov. 1996) using the word Tat yielded more than 1400 papers in the last three year period. Obviously, one can't cover all these new findings in this brief summary on this intriguing protein. It is hoped that this synopsis highlights some of the currently important issues on Tat, albeit considered in a rather subjective manner. I hope to update periodically the contents in this text in coming years and in coming editions of the database.

I conclude this writeup with a figure (figure 4, provided by Dr. E. P. Loret) showing recently derived computer models of Tat structure (Gregoire and Loret, 1996). As yet, a crystal structure for Tat is not available. However, analyses of multiple sequences do yield informative findings. For instance, these analyses reveal that the regions circumscribed by amino acids 38–47 and 59–72 have the widest degree of three-dimensional structural variability between different HIV-1 Tat proteins. The structure of other portions of Tat seems to be well-conserved. At the level of our current understanding of Tat function, it is difficult to establish a clear one-for-one correlation between the physical model (figure 4) and the functional phenotypes revealed by point mutants (Table 1). In coming years, I hope that this will change.



Tat BRU structure obtained from molecular modeling (Gregoire and Loret, 1996) using the atomic coordinates of the Tat Z2 2D-NMR structure (Bayer et al., *J. Mol. Biol*. **247**:529–535, 1995). Region I is colored in red, region II in orange, Region III in yellow, region IV in green, region V in light blue and region VI in blue. Molecular modeling was made with Insight II, Discover and Homology from MSI Technologies, Inc. (San Diego, CA) running on a R4600 Silicon Graphics Workstation. The CVFF force field was used to minimize the structure. Hydrogens were generated at pH 7. Steepest descent and conjugate gradient were the algorithms used for the minimization. Dynamic was performed at 300 K and 110 different structures were analyzed in the trajectory.



Backbone superimposition of Tat Bru (green) and Tat Oy (pink). The C-terminal extremity of Tat Oy (yellow) goes through the loop made by the cysteine rich region (region II) and ends up in a groove made by a part of region I and region III.

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